Author affiliations: National Reference Centre for Arboviruses and Hemorrhagic Fever Viruses, Aristotle University of Thessaloniki, Thessaloniki, Greece (A. Papa, A. Kontana); and Trikala General Hospital, Trikala, Greece (T. Paraforou, I. Papakonstantinou, K. Pagdatoglou, T. Koukoubani)

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Address for correspondence: Anna Papa, Department of Microbiology, Medical School, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece; email: email: annap@med.auth.gr

Phylogenetic Analysis of West Nile Virus Genome, Iran

To the Editor: West Nile virus (WNV) is a single-stranded, positive-sense RNA virus (≈11 kb) that is taxonomically classified within the family *Flaviviridae*, genus *Flavivirus*. WNV is found in Africa, Eurasia, Australia, and North America (*1*).

Comprehensive studies on phylogenetic relatedness of WNV strains have showed that WNV can be grouped into 5 lineages. Lineage 1 contains WNV strains from different regions, including northern, western, and central Africa; southern and eastern Europe; India; and the Middle East. Lineage 1 is subdivided into 3 clades. Clade 1A contains strains from Europe, northern Africa, the United States, and Israel, clade 1B contains Kunjin virus from Australia. Lineage 2 contains isolates from west, central, and eastern Africa and Madagascar. There is evidence that lineage 2 circulates in some regions of Europe (e.g., Italy, Austria, and Greece) (*2*,*3*). Lineage 3 contains Rabensburg virus 97–103, which was isolated in 1997 from *Culex pipiens* mosquitoes in South Moravia in the Czech Republic. Lineage 4 contains a new variant of WNV (strain LEIVKrnd88–190), which was isolated in 1998 from *Dermacentor marginatus* ticks in a valley in the northwestern Caucasus Mountains of Russia. Lineage 5 contains an WNV isolate from India (strain 804994) (*4*,*5*). In this study, we compared the phylogenetic relationships of WNV circulating in Iran to other WNV strains by using a partial WNV sequence isolated from an Iranian patient.

WNV was obtained from a blood sample from an Iranian patient who had encephalitis and was hospitalized in 2009 in Isfahan in the central highlands of Iran. The patient reported no history of animal contact, insect bites, blood transfusions, transplantations, and travel. He exhibited fever, headache, hypertension, and vomiting. On initial examination, he had a body temperature of 40°C. Laboratory investigations on the day of admission showed a leukocyte count of 240 cells/mL, a protein level of 52 mg/dL, and a glucose level of 50 mg/dL in a cerebrospinal fluid sample.

Further examinations were undertaken, and samples were sent to the Arboviruses and Viral Hemorrhagic Fevers Laboratory at Pasteur Institute of Iran in Teheran. For an IgG ELISA, wells in test plates were coated overnight with mouse hyperimmune ascitic fluid. Native antigen was added, and wells were incubated and washed. Test samples and peroxidase-labeled anti-human or anti-animal immunoglobulin were added. After incubation for 10 min, optical densities were read (*6*).

Viral RNA was extracted by using the QIAmp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) from serum of the patient. A reverse transcription PCR was conducted by using a One-Step RT-PCR Kit (QIAGEN). Samples were subjected to 1 cycle at 50°C for 30 min to synthesize cDNA; 95°C for 15 min; and 95°C for 30 s, 54°C for 30 s, and 72°C for 60 s; and a final extension at 72°C for 5 min (*6*). The serum sample was positive for IgG against WNV. Molecular tests showed positive results for WNV.

The PCR product was sequenced by using the Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied

Biosystems, Foster City, CA, USA), the modified Sanger sequencing method, and an ABI Genetic Analyzer 3130 (Applied Biosystems) (*7*). Multiple alignments of nucleotide sequences were made by using ClustalW (http://www.clustal.org/). A phylogenetic tree was constructed by using 27 representative sequences of WNV and Japanese encephalitis virus (available in GenBank) and a 358-nt sequence of WNV (GenBank accession no. KJ486150), from the patient, which corresponds to nt 259–616 in the late region of the capsid gene and the early region of the membrane gene. Phylogenetic status of the sequence for the WNV strain from the patient was assessed by using the neighbor-joining algorithm in MEGA5 (*8*). Reliability of phylogenetic groupings was evaluated by using the bootstrap test (1,000 replications). Japanese encephalitis virus SA14 sequence was used as an outgroup in phylogenetic analysis of partial genome sequences (*8*).

The phylogenetic tree identified clustering of isolates in 5 lineages. Lineage 1 had 2 sublineage (clade 1A and clade 1B). All sequences in lineage 1 were geographically distinguishable. Clade 1A contained strains from Europe, Africa, the Middle East, and the United States; clade 1B included the Australian strain NSW 2011 (JN887352). The WNV sequence from Iran (KJ486150) was grouped into lineage 2 and had 99% identity with the 358-bp region of WNV strain ArB3573/82 from the Central African Republic. Although it was believed that lineage 2 strains circulate only in Africa, reports of their emergence primarily in Balkan countries (*9*) and in our study support the presence of a lineage WNV 2 strain in the Middle East, particularly in western Asia (Figure).

Although the patient did not report any mosquito bites, this infection route cannot be excluded because he was a farmer and spent most of his time outdoors. Previous studies have demonstrated that nearly all human WNV infections were a consequence of mosquito bites (*10*). Our study should increase awareness of WNV infections as a public health threat. In future studies, priority should be given to investigations of the ecology, occurrence, and epidemiology of the different WNV strains circulating in Iran.

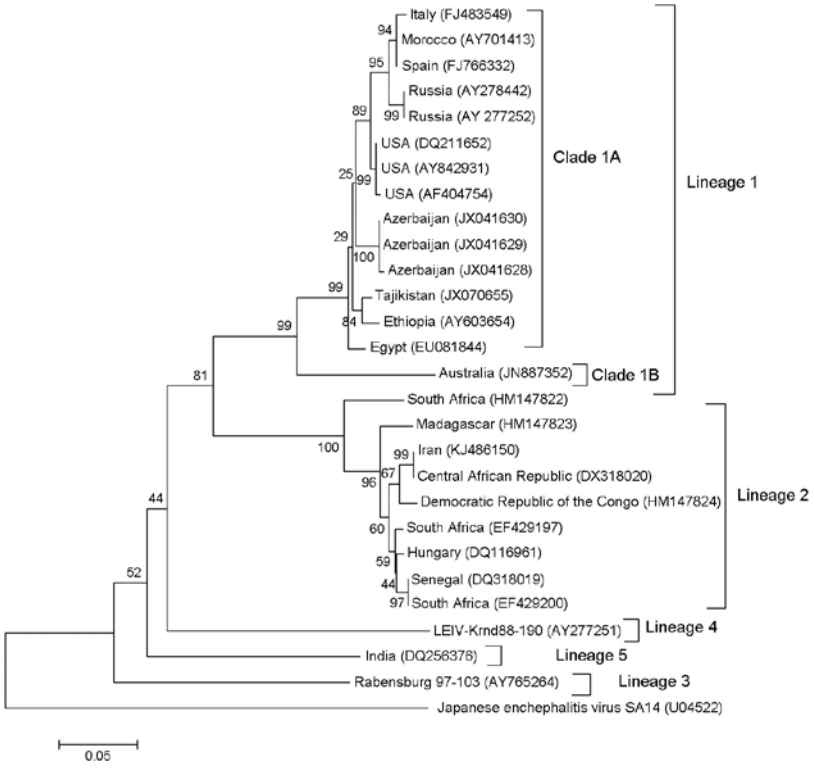

Figure. Phylogenetic tree based on a 358-nt sequence (nt 259–616) of 27 strains of West Nile virus (WNV) generated by using the neighbor-joining algorithm in MEGA5 (*8*). Japanese encephalitis virus was used as an outgroup. Location of virus isolation and GenBank accession numbers (in parentheses) are provided. The WNV sequence from Iran (KJ486150) was grouped into lineage 2 and had 99% identity with the 358-bp region of WNV strain ArB3573/82 from the Central African Republic. Scale bar indicates nucleotide substitutions per site.

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Nariman Shah-Hosseini, Sadegh Chinikar, Behroz Ataei, Anthony R. Fooks, and Martin H. Groschup

Author affiliations: Pasteur Institute of Iran, Tehran, Iran (N. Shah-Hosseini, S. Chinikar); Isfahan University of Medical Sciences, Isfahan, Iran (B. Ataei); Veterinary Laboratories Agency, Addlestone, UK (A.R. Fooks); and Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany (M.H. Groschup)

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Address for correspondence: Sadegh Chinikar, Laboratory of Arboviruses and Viral Haemorrhagic Fevers, Pasteur Institute of Iran, 60 Pasteur Ave, Teheran 1316943551, Iran; email: sadeghchinikar@yahoo.com

Human Infection with West Nile Virus, Xinjiang, China, 2011

To the Editor: West Nile virus (WNV) is a mosquito-borne flavivirus in the Japanese encephalitis serocomplex of the family *Flaviviridae* (*1*). It has been reported in Africa, Asia, Europe, Australia, and North America, and is recognized as the most globally widespread mosquito-borne flavivirus (*2*). Isolation of WNV has previously been attempted in China, Japan and South Korea; however, no virus has been isolated (*3*–*5*). We report isolation of WNVs from mosquitoes in Xinjiang Uyghur Autonomous Region in western China. We also provide evidence of WNV human infections confirmed by IgM ELISA and seroconversion by 90% plaque reduction neutralization tests of paired serum samples obtained from persons with febrile illness and viral encephalitis in 2011.

Arbovirus surveillance was performed in the Kashi Region, Xinjiang, China, in August 2011. Mosquitoes were captured by using light traps and gravid traps for 15 days (days 1–5, 11–15, and 21–25) at 9 collection sites in 9 villages in 2 townships. A total of 7,122 mosquitoes, representing 3 genera and 7 species in 118 pools, were tested. Mosquitoes collected were *Culex pipiens pipiens* (65.0%, 4,629/7,122), *Aedes flavidorsalis* (24.1%, 1,717/7,122), *Ae. caspius* (10.1%, 716/7,122), and other *Aedes* and *Culex* species (0.8%, 40/7,122) mosquitoes.

Mosquitoes were homogenized, and viral RNA was extracted directly from mosquito pools and amplified by using PCR and primers specific for WNV envelope (E) and nonstructural protein 5 genes as described (*6*). A total of 12 pools of *Cx. p. pipiens* mosquitoes were positive for WNV, which was confirmed by nucleotide sequencing. The minimum infection rate for *Cx. p. pipiens* mosquitoes was 2.56 infections/1,000 specimens tested.

In addition, supernatants of the 12 WNV-positive mosquito pools were inoculated onto Vero cells. Five pools yielded 5 virus isolates designated XJ11129–3, XJ11138–6, XJ11141–4, XJ11146–4, and XJ11148–2. The Vero cells aggregated and began shedding virus by 72 h postinfection.

Phylogenetic comparisons of complete nucleotide sequences of E gene from the 5 Xinjiang isolates (Figure, panel A) showed a high degree of genetic identity of lineage 1 with other highly pathogenic WNV strains, such as WNV NY99 and isolates from Russia. Nucleotide and amino acid sequences showed ≥99% identity with isolates from Russia (1999–2004) (*7*).

The complete nucleotide sequence of XJ11129–3 contained 11,029 nt, and the phylogenetic tree of the nucleotide coding region showed similar topology with the E gene tree (Figure, panel B). Nucleotide sequences of E genes from XJ11138–6, XJ11141–4, XJ11146–4, and XJ11148–2 and the complete genome sequence of XJ11129–3 were submitted to GenBank under accession nos. JX442280, JX442281, JX442282, JX442278, and JX442279, respectively.

To determine whether humans were infected with WNV, we obtained acute-phase serum samples within 1–7 days of onset of illness from persons visiting an outpatient clinic in Kashi during June 11–August 25, 2011. All patients had fever (37°C–39°C) or viral encephalitis with or without symptoms of encephalitis. Serum samples were obtained from 254 patients with fever of unknown origin and 9 patients with encephalitis.

All acute-phase serum samples were initially screened for IgM against WNV (WNV IgM Capture DxSelect; Focus Diagnostics Inc., Cypress, CA, USA) and against Japanese encephalitis virus (JEV) (JEV IgM Capture ELISA Kit; Panbio, Sinnamon Park, Queensland, Australia). A total of 38